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Registration Action Branch 1, Health Effects Division (7509C) **Date** 10/6/2004

TXR#: 0050344

DATA EVALUATION RECORD

STUDY TYPE: 28-Day Dermal Toxicity - Rat; OPPTS 870.3200 [§82-2]; OECD 410.

PC CODE: 112600 **DP BARCODE**: D278326
SUBMISSION NO.: S599442

TEST MATERIAL (PURITY): Prohexadione calcium (94.9% a.i.)

SYNONYMS: Calcium 5-oxo-4-propionylcyclohex-3-ene-3-olatecarboxylate (IUPAC)

CITATION: Mellert W, Deckardt K, Gembardt Ch, et al. (2001) Prohexadione Calcium - Repeated Dose Dermal Toxicity in Wistar Rats, Administration for 4 Weeks. BASF Aktiengesellschaft, Experimental Toxicology and Ecology, Rhine, Germany. Laboratory Project #: 33C0341/00059, May 8, 2001. MRID 45430301. Unpublished.

SPONSOR: BASF Corp., Agricultural Products, P.O. Box 13528, Research Triangle Park, NC

EXECUTIVE SUMMARY: In a 28-day dermal toxicity study (MRID 45430301), prohexadione calcium (94.9% a.i.; Batch#: FW 15175) in aqua bidest was applied to the shaved intact skin of 10 Wistar rats/sex/dose at dose levels of 0, 60, 300, or 1000 mg/kg bw/day, 6 hours/day for 5 days/week during a 28-day period.

No treatment-related effects were observed in mortality, clinical signs, dermal effects, body weight, body weight gain, food consumption, hematology, clinical chemistry, urinalysis, ophthalmology, organ weight, gross or histologic pathology at any dose in either sex.

The NOAEL is 1000 mg/kg/day (limit dose) in both sexes. The LOAEL was not observed.

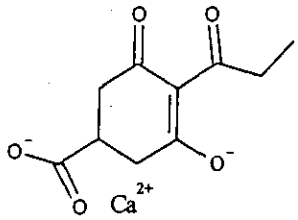
This study is classified as **Acceptable/Guideline** and satisfies the guideline requirement (OPPTS 870.3200; OECD 410) for a 28-day dermal toxicity study in rats.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material**: Prohexadione calcium
   Description: Light yellow solid. The test material was shown to be >95% stable in vehicle for at least 4 hours at room temperature.
   Batch #: FW 15175
   Purity (w/w): 94.9% a.i.
   CAS # of TGAI: 127277-53-6
   Structure:



2. **Vehicle:** Aqua bidest

3. **Test animals**
   Species: Rat
   Strain: Wistar [CRL:WI (GLX/BRL/HAN) IGS BR]
   Age/weight at dosing: 8-9 weeks old; 174.7-218.4 g (males) and 137.2-172.7 g (females).
   Source: Charles River, Sulzfeld, Germany
   Housing: Individually in Type DK III stainless steel wire-mesh cages
   Diet: Basic maintenance diet for mouse/rat, 9433 LL Meal (Eberle Nafag AG Gossau, Switzerland), *ad libitum*, except for 16-20 hrs. (fasting period) prior to blood collection and during urine collection.
   Water: Tap water, *ad libitum*, except during urine collection.
   Environmental conditions
   Temperature: 20-24°C
   Humidity: 30-70%
   Air changes: Not reported
   Photoperiod: 12 hrs. dark/12 hrs. light
   Acclimation period: 13-14 days

B. STUDY DESIGN

1. **In-life dates**: Start: 10/09/00 End: 11/08/00

2. **Animal assignment**: Animals were assigned randomly with respect to body weight (bw) to the test groups noted in Table 1.

Table 1. Study design [a].

Group	Dose (mg/kg bw)	# Male	# Female
Control	0	10	10
Low	60	10	10
Mid	300	10	10
High	1000	10	10

[a] Data obtained from Study Report, page 17.

3. **Dose selection rationale:** In a preliminary study performed by BASF in 2000, 3 Wistar rats/sex/dose were exposed dermally to 0 or 1000 mg/kg/day for 2 weeks. There were no effects of treatment on body weight, clinical signs, or gross pathology. Based on these results, the doses presented in Table 1 (above) were selected for the definitive study.

4. **Dosage preparation and analysis:** Dose formulations were prepared daily prior to dosing by suspending the appropriate amount of test substance in aqua bidest, based on the most recent body weight data. In a previous study (BASF 1994) using a different batch (G-14-37-114), the test material was shown to be stable in vehicle for at least 4 hours at room temperature. Measured concentrations across time for a given dose and homogeneity (the coefficients of variation across time for a given dose) were verified for all doses three days prior to dose initiation, at the end of Week 1, and at the termination of dosing.

Results

Stability Analysis (range of nominal concentration):
5% (w/v) suspension: 95.2%-100.8%

Concentration Analysis (range of % of nominal):
60 mg/kg/day: 95.7-105.8
300 mg/kg/day: 91.2-103.6
1000 mg/kg/day: 90.2-101.1

Homogeneity (range of CV%):
60 mg/kg/day: 0.5-3.0
300 mg/kg/day: 0.1-2.0
1000 mg/kg/day: 0.3-0.7

The analytical data indicated that the mixing procedure was adequate and the variation between nominal and actual dosage to the study animals was acceptable.

5. **Preparation and treatment of animal skin:** Twenty-four hours prior to the first application, and as needed thereafter, the fur of each test animal was clipped from the dorsal area of the trunk. A volume of 2 ml/kg of test material in vehicle was applied to the shaved skin (at least 10% of the total body surface), covered with 4 layers of a porous gauze pad, and held in place with an

elastic bandage. The dressings were removed after 6 hours, and the application areas were washed with warm water to remove any excess test material. Animals in the control group were exposed to vehicle only, using the same procedure as described for the treated animals.

6. **Statistics:** The following statistical methods were applied to the data:

Parameters Investigated	Statistical Test
Body weight Body weight gain Food consumption Food efficiency	Dunnett's (two-sided, $p \leq 0.05$ or 0.01)
Hematology (except differential blood count) Clinical chemistry Organ weights	Non-parametric, one way analysis of variance using Kruskal-Wallis test (two-sided, $p \leq 0.05$ or 0.01). Followed by Wilcoxon test if significant at $p \leq 0.05$.
Urinalysis (except for volume, appearance, and specific gravity)	Fisher's exact test ($p \leq 0.05$ or 0.01)

C. METHODS

1. Observations

a. Cage-side observations: All animals were examined twice daily (once daily on weekends and holidays) for signs of toxicity and mortality.

b. Clinical examinations: Detailed physical examinations were performed before treatment on Day 0 and weekly throughout the study. Examination of the skin was also performed daily prior to treatment (method not reported).

c. Neurological evaluations: Neurological evaluations were performed in a standard arena as part of the detailed clinical observations in this study. The following parameters were evaluated: behavior during handling, fur, skin, posture, salivation, respiration, activity/arousal level, tremors, convulsions, abnormal movements, impairment of gait, lacrimation, palpebral closure, exophthalmos, appearance/consistency of feces, urine, and pupil size. Motor activity, grip strength, and sensory reactivity to external stimuli were not measured.

2. Body weight: Animals were weighed prior to initiation of treatment (during randomization), on Day 0 (start of treatment), and weekly thereafter.

3. Food and water consumption: Individual food consumption was determined weekly, and mean food consumption was reported as g food/animal/day. Mean food efficiency (%) was determined weekly using individual body weight and food consumption data. Water consumption was observed daily by visual inspection, but no data were recorded.

4. Ophthalmoscopic examination: The eyes of all animals were examined 3 and 4 days before dosing in males and females, respectively, using a mydriatic agent and an ophthalmoscope. On Days 25 and 24 (in males and females, respectively) the eyes of the control and 1000 mg/kg animals were examined in a similar manner.

5. Hematology & clinical chemistry: After fasting for 16-20 hours, blood was collected from the retro-orbital venous plexus of all animals for hematology and clinical chemistry examinations. Differential blood smears were prepared, but not examined. The CHECKED (X) parameters were examined.

a. Hematology

X	Hematocrit (HCT)*	X	Mean corpusc. HGB conc. (MCHC)*
X	Hemoglobin (HGB)*	X	Mean corpusc. volume (MCV)*
X	Leukocyte count (WBC)*	X	Differential blood (leukocyte) count*
X	Erythrocyte count (RBC)*		Reticulocytes
X	Platelet count (PLT)*		
	Blood clotting measurements*		
X	(Prothrombin time)		
X	Mean corpuscular HGB (MCH)*		

* Recommended for 28-day dermal toxicity studies based on Guideline 870.3200

b. Clinical chemistry

	ELECTROLYTES		OTHER
X	Calcium	X	Albumin*
X	Chloride	X	Creatinine*
X	Magnesium	X	Urea nitrogen*
X	Phosphate	X	Cholesterol*
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
	ENZYMES	X	Total bilirubin
X	Alkaline phosphatase (ALP)*	X	Total protein*
	Cholinesterase (ChE)	X	Triglycerides
	Creatine phosphokinase		
	Lactic acid dehydrogenase (LDH)		
X	Alanine aminotransferase (ALT/also SGPT)*		
X	Aspartate aminotransferase (AST/also SGOT)*		
X	Gamma glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		
	Sorbitol dehydrogenase*		

* Recommended for 28-day dermal toxicity studies based on Guideline 870.3200

6. **Urinalysis**: Urine was collected by placing animals in metabolism cages overnight (without food and water). The CHECKED (X) parameters were examined.

X	Appearance (color and turbidity)	X	Glucose
X	Volume	X	Ketones
X	Specific gravity	X	Bilirubin
X	pH	X	Blood / blood cells
X	Sediment (microscopic)		Nitrate
X	Protein		Nitrites
	Leukocytes	X	Urobilinogen

7. **Sacrifice and pathology:** All surviving animals were sacrificed via CO_2 asphyxiation and subjected to gross pathological examination. The CHECKED (X) tissues were collected from all animals and preserved in 4% formaldehyde solution. The (XX) organs were also weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue	X	Aorta, thoracic*	XX	Brain*+
X	Salivary glands*	XX	Heart*+	X	Peripheral nerve (sciatic)*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	XX	Spleen*+	X	Eyes (optic nerve)*
X	Jejunum*	XX	Thymus*+		GLANDULAR
X	Ileum*			XX	Adrenal gland*+
X	Cecum*		UROGENITAL		Lacrimal gland
X	Colon*	XX	Kidneys*+	X	Parathyroid*
X	Rectum*	X	Urinary bladder*	X	Thyroid*
XX	Liver*+	XX	Testes*+		OTHER
	Gall bladder* (not rat)	XX	Epididymides*+	X	Bone (femur)
	Bile duct* (rat)	X	Prostate*	X	Skeletal muscle
X	Pancreas*	X	Seminal vesicles*	X	Skin* (treated & untreated)
	RESPIRATORY	XX	Ovaries*+	X	All gross lesions*
X	Trachea*	X	Oviducts	X	Extraorbital lacrimal glands
X	Lung*	XX	Uterus*+		
X	Nose*	X	Mammary gland*		
X	Pharynx*	X	Vagina		
X	Larynx*				

* Required for 28-day dermal toxicity studies based on Guideline 870.3200
\+ Organ weights required.

All organs and tissues from the control and high-dose animals, except skeletal muscle and the extra-orbital lacrimal glands, were preserved in 4% formaldehyde solution, embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined by light microscopy. Any gross lesions from all other animals were also examined microscopically.

II. RESULTS

A. OBSERVATIONS

1. **Clinical signs:** No treatment-related clinical signs were observed in any animal in either sex. Motor activity, grip strength, and sensory reactivity to extemal stimuli, however, were not measured.

2. **Mortality:** All animals survived to scheduled necropsy.

3. **Dermal irritation:** No treatment-related dermal effects were observed at any dose in either sex.

B. BODY WEIGHT AND WEIGHT GAIN: No treatment-related effects on body weight or body weight gain were observed at any dose in either sex (Table 2).

Table 2. Mean (±SD) body weights in rats dermally exposed to prohexadione calcium for up to 28 days [a].

Dose (mg/kg)	Body Weights (g±SD)					Total Weight Gain	
	Day 0	Day 7	Day 14	Day 21	Day 28	g	% of control
Male							
0	199.1±9.2	222.6±11.0	240.6±14.66	253.6±17.7	262.1±20.9	63.0±19.1	100.0
60	202.0±10.4	222.7±11.5	243.3±13.9	258.7±17.2	265.2±17.6	63.2±12.5	100.3
300	196.2±11.2	219.6±7.4	239.3±6.8	251.6±9.4	258.3±14.2	62.1±17.8	98.6
1000	199.6±10.6	221.4±13.4	245.9±16.2	259.8±18.9	269.7±19.7	70.1±12.4	111.3
Female							
0	154.5±7.1	165.2±8.8	179.8±10.8	186.4±13.6	197.2±10.3	42.7±8.1	100.0
60	156.8±10.1	166.2±11.8	181.3±16.9	190.8±12.3	197.1±19.1	40.3±22.4	94.3
300	152.6±11.6	164.2±9.8	176.4±15.0	190.1±13.8	197.3±15.3	44.8±10.5	104.9
1000	155.1±9.7	166.0±11.3	180.5±11.9	188.9±14.5	197.1±15.9	42.0±9.0	98.4

[a] Data obtained from the Study Report, Table IA, pages 53-56; n=10.

C. FOOD AND WATER CONSUMPTION: No treatment-related effects on food consumption or food efficiency were observed at any dose in either sex. A general, non-adverse decrease in food efficiency in the males was observed across time in all doses. A significant increase (30%, $p \leq 0.05$) in food efficiency was noted on Day 14 in the 1000 mg/kg males and was

considered incidental and not adverse. No overt deviations in volume of water in the bottles were observed in treated groups compared to controls.

D. OPHTHALMOSCOPIC EXAMINATION: No treatment related effects were observed in the eyes of any animal in either sex.

E. BLOOD ANALYSES

1. Hematology: No treatment-related effects were observed in any hematological parameter at any dose in either sex.

2. Clinical chemistry: No treatment-related effects were observed in any clinical chemistry parameter at any dose in either sex.

F. URINALYSIS: No treatment-related effects were observed in any urinalysis parameter at any dose in either sex. Incidence of blood (>2) in the urine was increased (50%, $p \leq 0.05$) compared to controls in the males at 300 mg/kg; however, this finding was considered unrelated to treatment, because this response was not dependent on dose. [High-dose males had an incidence of 3/10 for blood in the urine.] Incidence of blood in the urine was also slightly increased in females in the high-dose group (4/10) above the controls (1/10) and low-dose females (1/10). Histological examination of the kidneys revealed no treatment related lesions.

G. SACRIFICE AND PATHOLOGY

1. Organ weight: In females, increases ($p \leq 0.05$) in absolute brain weight were observed at 300 (↑4%) and 1000 (↑4%) mg/kg compared to controls; however, this finding was considered unrelated to treatment because the increases were minor and there was no corroborative gross or histological data. Similarly, the increased absolute adrenal weights observed in the 300 mg/kg females (↑21%) were considered unrelated to treatment, since there was no dose-response effect or corroborative gross or histological data. No other treatment-related changes in organ weight were observed. No treatment-related effects were observed in any relative (to body) organ weights at any dose in either sex.

2. Gross pathology: No treatment-related gross lesions were observed at any dose in either sex.

3. Microscopic pathology: No treatment-related histopathological findings were observed at any dose in either sex.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: The investigators concluded that under the conditions of this study, no treatment-related effects were observed in any measured parameters in rats dermally exposed to prohexadione calcium five days/week for four weeks at up to1000 mg/kg (limit dose). The NOAEL was 1000 mg/kg/day.

B. REVIEWER COMMENTS: No treatment-related effects were observed in mortality, clinical signs, dermal effects, body weight, body weight gain, food consumption, hematology, clinical chemistry, urinalysis, ophthalmology, organ weight, gross or histologic pathology at any dose in either sex. Motor activity, grip strength, and sensory reactivity to external stimuli were not measured in week 3 or 4, as required by OPPTS 870.3200 (1998). However, the results of all other clinical observations argue against any potential adverse effects on these specific endpoints.

The NOAEL is 1000 mg/kg/day (limit dose) in both sexes. The LOAEL was not observed.

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirement (OPPTS 870.3200; OECD 410) for a 28-day dermal toxicity study in rats.

C. STUDY DEFICIENCIES: The following minor deficiencies were noted, but do not change the results of this review:

- 1. The method of dermal evaluation was not provided.
- 2. Motor activity, grip strength, and sensory reactivity to external stimuli were not measured in week 3 or 4, as required by OPPTS Guideline 870.3200 (1998).
- 3. Individual animal food efficiency calculations were not provided.

DATA FOR ENTRY INTO ISIS

Subchronic Dermal (28 day) Study - rats (870.3200)

PC code	MRID #	Study type	Species	Duration	Route	Dosing method	Dose range mg/kg/day	Doses tested mg/kg/day	NOAEL mg/kg/day	LOAEL mg/kg/day	Target organ(s)	Comments
112600	45430301	subchronic	rat	28 days	dermal	dermal	60-1000	0, 60, 300, 1000	1000	Not observed		Systemic
112600	45430301	subchronic	rat	28 days	dermal	dermal	60-1000	0, 60, 300, 1000	1000	Not observed		Dermal